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A First-in-Man, Randomized, Placebo-Controlled Study to Evaluate the Safety and Feasibility of Autologous Delipidated High-Density Lipoprotein Plasma Infusions in Patients With Acute Coronary Syndrome

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Objectives This study aimed to determine whether serial autologous infusions of selective high-density lipoprotein (HDL) delipidated plasma are feasible and well tolerated in patients with acute coronary syndrome (ACS).

Background Low HDL is associated with increased risk of cardiovascular disease. Plasma selective delipidation converts αHDL to preβ-like HDL, the most effective form of HDL for lipid removal from arterial plaques.

Methods ACS patients undergoing cardiac catheterization with ≥1 nonobstructive native coronary artery atheroma were randomized to either 7 weekly HDL selective delipidated or control plasma apheresis/reinfusions. Patients underwent intravascular ultrasound (IVUS) evaluation of the target vessel during the catheterization for ACS and up to 14 days following the final apheresis/reinfusion session. 2-D gel electrophoresis of delipidated plasmas established successful conversion of αHDL to preβ-like HDL. The trial was complete with 28 patients randomized.

Results All reinfusion sessions were tolerated well by all patients. The levels of preβ-like HDL and αHDL in the delipidated plasma converted from 5.6% to 79.1% and 92.8% to 20.9%, respectively. The IVUS data demonstrated a numeric trend toward regression in the total atheroma volume of -12.18 ± 36.75 mm^3 in the delipidated group versus an increase of total atheroma volume of 2.80 ± 21.25 mm^3 in the control group ($p = 0.268$).

Conclusions In ACS patients, serial autologous infusions of selective HDL delipidated plasma are clinically feasible and well tolerated. This therapy may offer a novel adjunct treatment for patients presenting with ACS. Further study will be needed to determine its ability to reduce clinical cardiovascular events. (J Am Coll Cardiol 2010;55:2727–35) © 2010 by the American College of Cardiology Foundation

Over the last decade, clinical trials of low-density lipoprotein cholesterol (LDL-C)-lowering drugs have definitively established that reductions in LDL-C are associated with a 30% to 45% decrease in clinical cardiovascular disease (CVD) events (1–5). However, despite lowered LDL-C, many subjects continue to present with cardiac events. Low high-density lipoprotein cholesterol (HDL-C) is often present in high-risk subjects with CVD (6,7), and epidemiological studies have identified HDL-C as an independent risk factor that modulates CVD risk (8–10). In addition, other lines of evidence suggest that raising HDL-C would reduce the risk of CVD. Infusion of HDL is associated with regression of atherosclerosis in cholesterol-fed rabbits (11). Furthermore, increased plasma HDL-C concentrations achieved by overexpressing human apoA-I in transgenic mice (12,13), and human cholesterol acyltransferase in transgenic rabbits protect against the development of atherosclerosis (14). A summary of clinical trials that evaluated the potential efficacy of combined LDL reduction with HDL elevation provides additional support in that increasing HDL reduces cardiovascular clinical events (15). Angiographic imaging trials using intravascular ultrasound (IVUS) have indicated that increasing HDL is

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Abbreviations and Acronyms

ACS = acute coronary syndrome
CVD = cardiovascular disease
EEM = external elastic membrane
HDL = high-density lipoprotein
HDL-C = high-density lipoprotein cholesterol
IVUS = intravascular ultrasound
LDL = low-density lipoprotein
LDL-C = low-density lipoprotein cholesterol

associated with a regression of atherosclerosis and that a 7.5% increase in HDL is associated with regression in atherosclerosis (16). Similarly, in an imaging trial utilizing carotid intima-media thickness, the addition of niacin to statin-treated patients resulted in the regression of atherosclerosis (17). It has been concluded from the imaging trials that statin therapy is effective in reducing the progression of atherosclerosis; however, increasing HDL is required for extensive regression of disease. These combined results have provided substantial support for increasing HDL as a target to reduce atherosclerosis in the high-risk patient with CVD.

A major advance in the clinical development of HDL therapy for high-risk CVD subjects is the apoA-I Milano clinical trial in subjects with acute coronary syndrome (ACS). In this trial, subjects received 5 weekly infusions of a synthetic form of preβ-HDL, apoA-I Milano/phospholipid complex. Study results established that selectively increasing preβ-HDL was associated with regression of total atheroma volume by 4.2% in 36 subjects compared with 11 controls utilizing IVUS to quantitate coronary atheroma (18). These results provide support to the concept that selectively increasing preβ-HDL represents a new therapeutic target for prevention of CVD. The purpose of this study was to examine the safety of the Lipid Sciences Plasma Delipidation System-2 (LS PDS-2) investigational device with hypothesis-generating exploration of the potential impact of this therapy on atheroma volume measured by IVUS.

Methods

The current LS-001 (Lipid Sciences Selective Delipidation Trial) study is a randomized, single-blind, placebo-controlled study to evaluate the safety of the LS PDS-2 device in subjects who had been previously treated for ACS. Patients with ACS scheduled for cardiac catheterization with a nonobstructive atheroma in ≥1 native coronary arteries were randomized to HDL delipidation or control, and subjected to apheresis/reinfusion. Patients had 7 sessions each, 1 week apart. Patients underwent IVUS evaluation of the target vessel during the diagnostic catheterization for ACS and up to 14 days following the final procedure. The study was conducted from June 2006 to February 2008 under approval of the Food and Drug Administration and the institutional review board at Washington Hospital Center; with written informed consent obtained from patients prior to any study-related procedures. An independent clinical event committee adjudicated adverse events.

Study population. Patients age 18 to 85 years who presented with ACS (defined as unstable angina or non–ST-segment elevation myocardial infarction) who were scheduled for a diagnostic catheterization, who had a left ventricular ejection fraction >40%, and did not require insulin for diabetes were eligible for screening in this study. Subjects must have had angiographic evidence of coronary heart disease defined as having ≥1 lesion that had ≥20% but ≤50% reduction in lumen diameter by visual estimation within a segment 30 to 80 mm in length in a native coronary artery that did not require percutaneous coronary intervention (PCI) and without a bypass graft. A single artery was identified as the target artery for each subject and remained constant throughout the study. Patients were considered eligible for this study if they also met normal inclusion requirements for plasmapheresis: including weighing 110 lbs or more, hemoglobin (≥12.5 g/dl), with sufficient levels of HDL-C (≥32 mg/dl) and apoA-I (≥95 mg/dl), as well as triglycerides <300 mg/dl.

Randomization and binding. Subjects who met the eligibility requirements and had been discharged from the ACS hospitalization underwent a 7-week investigational treatment period. These treatments began within 14 days following the admission of ACS. Upon return to the clinic, subjects were randomized in a 1:1 fashion to either selective HDL delipidation utilizing the LS PDS-2 device or control where no delipidation treatment of the plasma occurred. All treatments followed the same randomization assignment, and cross-over was not allowed. The trial was conducted in a single-blind fashion in which the patients were unaware of their randomization assignment throughout the trial.

Plasma collection, delipidation, and reinfusion. Approximately 1 l of plasma was collected by apheresis from each patient in an identical manner via the Baxter Auto-C Plasmapheresis System (Fenwal, Lake Zurich, Illinois). All patients received a minimum of 500 ml of 5% human albumin with additional saline as needed per clinical judgment of the plasmapheresis operators. The 1 l of plasma was either subjected to the selective HDL delipidation process or was left untreated and reinfused back into the patient. The typical lengths of the initial plasmapheresis and reinfusions were 1.5 and 2 h, respectively. A 4-F catheter utilizing either the arm or groin as the access site was used for the plasmapheresis and reinfusions.

The delipidation process was performed using the investigational LS PDS-2 device designed to selectively remove cholesterol from HDL in plasma. This selective cholesterol removal, or delipidation, is achieved by mixing the collected plasma with organic solvents, *n*-butanol and sevoflurane, within the device. The used solvents and removed lipids are then separated from the plasma by gravity phase separation. The solvent/lipid mixture is drained, and the delipidated plasma is subsequently pumped through a charcoal column to remove trace solvent residual in the plasma (19). During

this process, the delipidated plasma is chased with approximately 150 ml of saline into a commercially available intravenous bag, aseptically moved from the PDS-2 system, and returned to the patient via a standard infusion pump. If randomized to the placebo control, the plasma was transferred to a 2.0-l return bag (as used for the delipidated group) with approximately 150 ml of saline, and returned to the patient via an infusion pump. The reinfusion intravenous return bags were identical for the investigational and placebo-control treatment groups to maintain the blind. The delipidated or control plasma was reinfused into the patient over an approximately 60- to 75-min period, with an observation period of 2 h after the completion of the reinfusion to assess for adverse effects. The patients were then released from the clinic.

The reinfusion of autologous selective delipidated HDL or control plasma was repeated every seventh day (±2 days) for a total of 7 treatment procedures. If a patient was unable to return for 1 of the 7-week clinic visits for investigational treatment within the allotted treatment window, or the full course of both the plasmapheresis and reinfusion treatment was unable to be completed, the visit was allowed to be rescheduled for the following week. This was allowed only once per patient, and only 7 completed treatment visits were allowed per patient.

All medications with the exception of clopidogrel were withheld on the day of plasmapheresis/reinfusion. Medications were to be resumed upon completion of release from the clinic. Antihypertensive medications were held at least 24 h prior to the plasmapheresis procedure. If the administration of antihypertensive medications was not able to be interrupted, the treating physician was asked to consider a decreased dosage on the treatment days. All patients were discharged on cholesterol-lowering medications following the ACS episode.

Safety indexes. Vital signs were monitored prior to the start of apheresis and prior to the reinfusion session, with assessment every 10 to 15 min during the reinfusion and

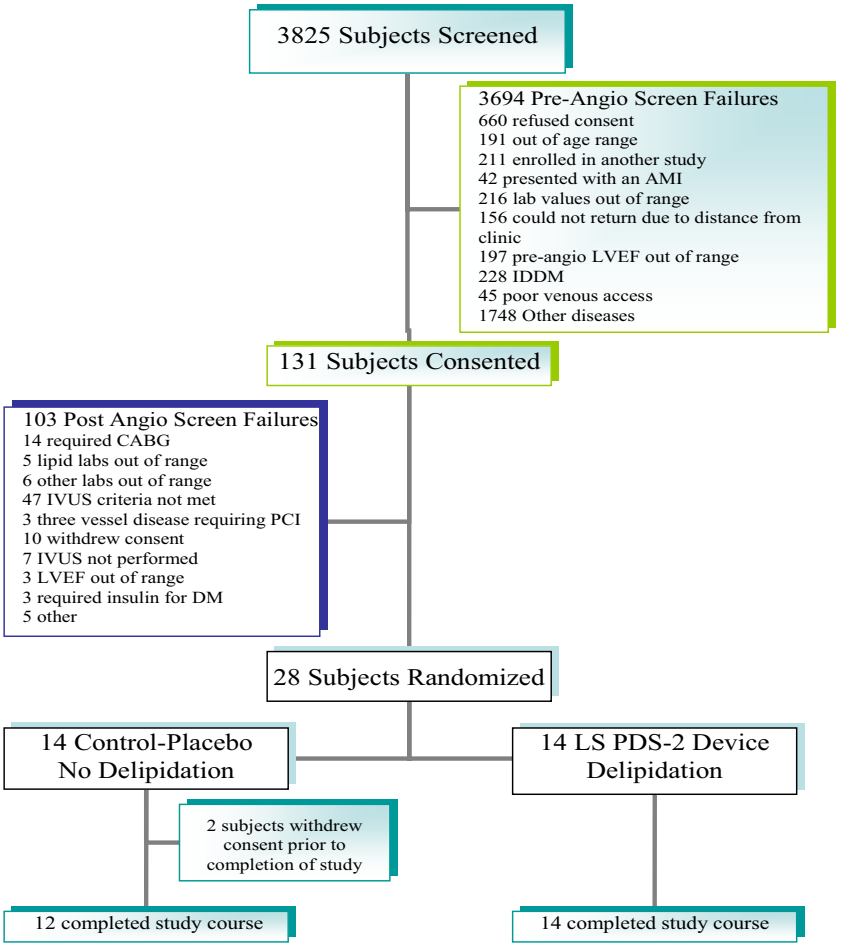

Figure 1 **Breakdown of the Study Population**

Schematic overview of the disposition of patients in the clinical trial. AMI = acute myocardial infarction; CABG = coronary artery bypass grafting; DM = diabetes mellitus; IDDM = insulin-dependent diabetes mellitus; IVUS = intravascular ultrasound; LS PDS-2 = Lipid Sciences Plasma Delipidation System-2; LVEF = left ventricular ejection fraction.

throughout the observation period. Clinical definitions for adverse events included bradycardia heart rate <40 beats/min, tachycardia heart rate >100 beats/min, and hypotension blood pressure <80/40 mm Hg. Laboratory values were assessed during screening, prior to apheresis, and following the completion of reinfusion at each clinic visit, prior to the repeat angiographic and IVUS evaluation, as well as during the final clinic visit. Laboratory values were assessed at all visits, including a complete blood count with differential and reticulocytes, chemistry panel, lipid profile including ApoA-I and ApoB, and coagulation panel as well as cardiac isoenzymes (creatine kinase myocardial band and troponin I), prior to the first clinic session of apheresis and reinfusion as well as at the final visit. Comparisons between pre-apheresis and post-reinfusion for each successful reinfusion visit were performed to observe frequency of transient changes with overall comparisons between pre-initial apheresis/reinfusion clinic visit and the final visit for sustained observations.

Quantitative 2-D gel electrophoresis and preβ-HDL (enzyme-linked immunoadsorbent assay [ELISA]) following delipidation. In 6 patients, plasma was collected post-delipidation or control treatment. Preβ-HDL was assessed by 2-gel electrophoresis and ELISA quantitation as previously described. The increase in ABCA1-mediated cholesterol in plasma following selective HDL delipidation was ascertained by utilizing in vitro cell culture studies as previously reported (19).

IVUS core laboratory analyses. Following diagnostic coronary angiography, the operator selected a single major epicardial vessel for interrogation based on the criteria previously reported. After anticoagulation and administration of 100 to 300 μg of intracoronary nitroglycerin, a 0.014-inch guidewire was subselectively placed in the vessel chosen for interrogation. A 40-MHz, 2.6-F (0.87-mm) IVUS catheter (Atlantis, Boston Scientific Scimed, Maple Grove, Minnesota) was advanced into the target vessel with the transducer positioned just distal to a side branch. The IVUS catheter was attached to a motorized pullback apparatus (at a speed of 0.5 mm/s) and to a dedicated ultrasound scanner (Clearview, Boston Scientific Scimed). During the pullback, IVUS images were obtained digitally at >30 frames/s, and the pullback lengths were required to be from 30 to 80 mm of the target vessel. At follow-up, the operator placed the IVUS catheter in the same vessel originally imaged, positioned the transducer just distal to the original branch, and initiated a motorized pullback. This procedure ensured that the identical segment was analyzed at baseline and follow-up.

The IVUS pullbacks were analyzed independently as pairs by an IVUS-trained technician and an experienced cardiologist who was blinded to the randomization treatment and temporal sequence of the imaging runs. The primary imaging analysis was conducted at the MedStar Research Institute IVUS Core Laboratory, and the methodology validating core laboratory was the IVUS Core Laboratory at Stanford University.

IVUS MEASUREMENTS. The methods for analysis have been previously described and were performed in accordance with the standards of the American College of Cardiology and European Society of Cardiology (20–22). For each cross-section, the operator performed manual planimetry to trace the leading edges of the luminal and external elastic membrane (EEM) borders. The maximum atheroma thicknesses were also directly quantitated. Atheroma area was calculated as EEM area minus luminal area. Image cross-sections were obtained at 0.25-mm intervals; the total atheroma volume was calculated using the Simpson rule as the sum of the atheroma area multiplied by interval length. The percent atheroma volume was also computed as:

$$\frac{\sum(\text{atheroma areas} \times \text{interval length})}{\sum(\text{EEM areas} \times \text{interval length})} \times 100$$

Additional IVUS measurements included the change in the plaque burden and the change in 10-mm most diseased and least diseased subsegment atheroma volume (mm^3).

Statistical analysis. Comparison between treatment groups was performed by nonparametric analysis with the Fisher exact test for categorical data, the Wilcoxon rank sum test for continuous data, and the Wilcoxon signed rank test for the evaluation of paired differences. Additional comparisons were made at the pre- and post-reinfusion safety data points for the entire cohort.

Results

Study population. From June 2006 to February 2008, 3,825 patients were screened for inclusion in the study. Twenty-eight subjects were enrolled in the study. Twenty-six of the 28 subjects enrolled completed all 10 visits—14 in

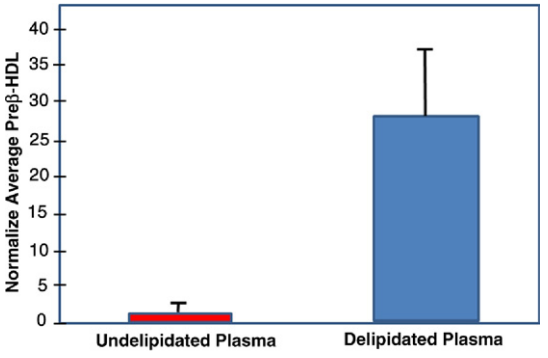

Figure 2 **Preβ-HDL in Post-Delipidated Plasma Increases an Average of 28× Versus Preβ-HDL in Pre-Delipidated Plasma**

Enzyme-linked immunoadsorbent assay quantitation of preβ-HDL in undelipidated plasma from control subjects (n = 6) and plasma for patients (n = 6) following high-density lipoprotein (HDL) selective delipidation.

Table 1 Baseline Characteristics of All Patients Randomized

Evaluated at Screening	Delipidation Group (n = 14)	Control Group (n = 14)
Age, yrs	55 (44–68)	55 (37–74)
Men	10 (71.4%)	12 (85.7%)
Diabetes	6 (42.9%)	2 (14.3%)
History of hypertension	10 (71.4%)	13 (92.9%)
History of hyperlipidemia	12 (85.7%)	13 (92.9%)
Current smoker	5 (35.7%)	6 (42.9%)
Prior percutaneous coronary intervention	4 (28.6%)	6 (42.9%)
Prior coronary artery bypass graft	1 (7.4%)	2 (14.3%)
Weight, kg	88 (68–108)	88 (58–117)
Statin use at randomization	12 (85.7%)	13 (92.9%)
Nonstatin cholesterol-lowering medication at randomization	2 (14.3%)	1 (7.4%)

Values are presented as median (range) or n (%).

the delipidation arm and 12 in the control arm (Fig. 1). Two subjects required the use of the protocol-allowed eighth clinic visit to complete the required 7 plasmapheresis/reinfusion visits. Two subjects withdrew consent during the trial prior to completion of all visits. One subject withdrew consent after visit 1, and the second withdrew consent after missing visit 7 and did not return to the clinic for this visit. There were no adverse events related to the cessation of the treatment in these 2 subjects. The median subject age was 55 years (range 37 to 74 years). Of the 28 subjects, 6 (21.4%) were female and 22 (78.6%) were male. Twenty-six patients completed IVUS within the 14 days after the final reinfusion (Fig. 2). The demographic baseline clinical characteristics of participants are summarized in Table 1.

Angiographic data. Twenty of the 28 subjects underwent PCI of a non-target vessel prior to enrolment at the time of the ACS screening hospitalization. The target artery was the right coronary artery for 11 (39.3%) of the subjects, the left anterior descending in 10 (35.7%), and the left circumflex in 7 (25.0%).

Clinical laboratory evaluation. There were no clinically associated differences in the laboratory indexes in comparison of pre-apheresis to post-reinfusion at any of the apheresis/reinfusion clinic visits. In addition, there were no clinically or statistically significant differences in the laboratory indexes when comparing the pre-initial plasmapheresis/reinfusion and the final visit values for each laboratory test for each subject (Tables 2 and 3).

ADVERSE EVENTS. Adverse events were monitored from the time of randomization through the final follow-up visit. Adverse events are noted in Tables 4 and 5. Fifteen of the 28 subjects experienced ≥1 adverse event during the study. The number of subjects experiencing adverse events in this study was equal between treatment groups ($p = 0.705$). In those 15 subjects, 38 adverse events were reported. All adverse events were adjudicated for relation to the plasmapheresis and reinfusion procedures by an independent clinical event committee. There was no statistical difference noted in frequency of adverse events between groups. Three patients underwent angioplasty due to progressive disease in a different non-target vessel at the time of repeat angiography/IVUS visit: 2 from the control, nondelipidated group and 1 in the delipidated group (Table 4). There were no reports of toxicity due to the solvents used during the delipidation procedure. The most common adverse event noted was hypotension related to the apheresis procedure. There were no serious adverse events related to reinfusion, and all subjects responded positively to conservative fluid supplementation therapy. The median total duration of plasmapheresis for the visits where hypotensive events occurred was 75 min (range 64 to 174 min); this was not different from the duration of apheresis seen in the rest of the patients. There were 2 laboratory reports of elevated potassium levels in the delipidated group and 1 in the control group; however, none of these reports was associated with any clinical symptoms of hyperkalemia, and repeat laboratory analysis did not demonstrate sustained elevations.

2-D gel electrophoresis and quantitative preβ-HDL (ELISA) analyses following delipidation. The PDS-2 selective HDL delipidation of human plasma consistently converts αHDL to preβ-like HDL. Preβ-like HDL in post-delipidated plasma increased an average of 28 times more than the baseline pre-delipidated plasma when analyzed using a quantitative preβ ELISA. Using 2-D gel electrophoresis, a large increase in HDL with preβ mobility

Table 2 Clinical Laboratory Data for Those Patients Who Completed All Clinic Visits

	Delipidation Group (n = 14)		Control Group (n = 12)		
	Pre	Final Visit	Pre	Final Visit	p Value*
Hemoglobin	14.1 (12.2–15.7)	13.8 (11.8–15.3)	14.1 (10–15.9)	13.6 (10.2–14.9)	0.315
Hematocrit	41.5 (37.5–44.7)	39.7 (37.0–43.2)	41.9 (31.3–45.9)	40.5 (31.4–43.6)	0.396
AST	27.5 (19–69)	28.5 (21–51)	24.5 (16–82)	23.5 (16–66)	0.367
ALT	38.5 (28–101)	36 (28–71)	40.5 (25–98)	35.5 (21–52)	0.777
Alkaline phosphatase	86 (61–132)	91 (62–129)	88 (65–163)	92.5 (60–135)	0.156
LDH	497.5 (336–708)	468.5 (354–804)	502.5 (353–696)	476.5 (367–787)	0.738
Total CPK	96.5 (31–233)	106 (40–288)	101.0 (20–314)	81.5 (20–1999)	0.625
Potassium	4.5 (3.9–5.6)	4.4 (3.9–4.9)	4.2 (4.0–5.3)	4.3 (3.9–7.5)	0.093

*Wilcoxon signed rank sum test.
ALT = alanine aminotransferase; AST = aspartate aminotransferase; CPK = creatine phoshosphokinase; LDH = low-density lipoprotein.

Table 3 Lipid Profile Data for All Patients Who Completed All Clinic Visits

	Delipidation Group (n = 14)		Control Group (n = 12)		
	Pre	Final Visit	Pre	Final Visit	p Value*
Total cholesterol	148.5 (106–246)	147 (90–203)	148 (121–212)	148.5 (99–221)	0.643
HDL-C	47 (33–65)	44 (34–59)	41.5 (32–82)	44 (27–86)	0.395
LDL-C	75 (45–133)	67.5 (35–120)	78 (43–113)	70.5 (40–131)	0.268
VLDL-C	20.5 (12.5–55.6)	27.5 (15.5–46)	24.2 (3–54.1)	28.3 (4.5–63.4)	0.662
ApoB	82.5 (55–123)	74 (46–105)	71 (57–100)	73 (46–116)	0.099
ApoA-I	139.1 (100.6–165.4)	129.1 (107.2–158.9)	126.4 (108.8–187.7)	124.5 (87.5–193.5)	0.554
Triglycerides	138.0 (65–446)	146.5 (66–360)	187.5 (65–505)	161.5 (64–440)	0.700

*Wilcoxon signed rank sum test.
Apo = apolipoprotein; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very low-density lipoprotein cholesterol.

was repeatedly observed (5.6% to 79.1%, respectively); whereas HDL with α mobility was repeatedly reduced from 92.8% in the undelipidated plasma to 20.9% post-delipidation (Fig. 2).

IVUS analysis. IVUS was a secondary end point collected for the purpose of exploration and to constitute pilot data from which to form the bases for a sample size calculation for a definitive pivotal study. Of the 28 patients enrolled into the trial, 26 underwent IVUS interrogation procedures with analyzable data at both time points. The average pull-back length was 33.6 ± 10.4 mm in the overall population, with no difference noted between the 2 groups. The change in mean total atheroma volume in the delipidated group was −12.2 ± 36.8 mm^3 from baseline to follow-up, whereas in the control group, it was 2.8 ± 21.3 mm^3 (p = 0.268). The mean change in plaque burden in the delipidated group was −1.0 ± 4.0% compared with baseline, whereas, on average, there was no change from baseline in the control group 0.0 ± 4.0% (p = 0.455). To determine the interaction between observed delipidation effects and disease severity, the protocol pre-specified analysis of the most severely and least severely diseased 10-mm-long subsegments. For the combined treatment cohort, the effect of delipidation was observed with increased numerical regression of disease in the most severely diseased 10-mm subsegment when compared with control, −6.6 ± 17.9 mm^3 versus −1.7 ± 11.2 mm^3, respectively, p = 0.494. In the least severely diseased subsegment, less of a numeric difference was observed when comparing the delipidation group to the control group (−1.1 ± 11.4 mm^3 vs. 1.5 ± 11.7 mm^3, p = 0.417) (Tables 6 and 7).

Table 4 Major Adverse Cardiac Events*

Variable	Delipidation Group (n = 14)	Control Group (n =14)
Death	0	0
Reinfarction	0	0
Target lesion revascularization	0	0
Nontarget lesion revascularization	1 (7.2%)	2 (11.8%)
Unanticipated adverse device effects	0	0

Variables n (%). *Intention to treat population.

Discussion

A pivotal mechanism by which HDL may protect against CVD is via the reverse cholesterol transport mechanism, a process by which HDL removes excess cholesterol from lipid-loaded macrophages and transports this cholesterol to the liver where any excess cholesterol can be excreted from the body as bile acids and cholesterol (23). The major pathway for cholesterol efflux from cholesterol-filled macrophages to HDL is by interaction of the newly synthesized disc-shaped nascent or preβ form of HDL with the ABCA1 transporter (24–27). Preβ-HDL is converted to spherical mature HDL, αHDL, following esterification of free cholesterol to cholesterol esters by lecithin:cholesterol acyltransferase. αHDL, which is the major form of HDL in plasma, does not interact with the ABCA1 transporter, the major pathway for cholesterol efflux from cells (28). αHDL, however, does facilitate cholesterol efflux from cells by interaction with 2 additional macrophage receptors, the SR-B1 receptor (29,30) and the ABCG1 transporter (31,32). After accepting excess cellular cholesterol from arterial macrophages and other peripheral tissues, αHDL transports the excess cholesterol to the liver.

Of particular interest with regard to the development of HDL therapy for the high-risk patient with CVD is the IVUS clinical trial with infusions of apoA-I Milano in ACS patients. The significant regression of atherosclerosis with

Table 5 Adverse Events

Variable	Delipidation Group (n = 14)	Control Group (n = 14)
Intermittent/permanent right bundle branch block	2	0
Hyperkalemia (by laboratory assay only)	2	1
Bradycardia	0	2
Tachycardia	0	2
Stable angina	0	1
Peptic ulcer	1	0
Irregular heart beat by electrocardiogram	0	1
Electrocardiogram abnormalities, by core laboratory	1	0
Hypotension	4	5

Values are adjudicated related to either apheresis or reinfusion procedures.

Table 6 IVUS Parameters, With Change Defined as Post-Delipidation Treatments Minus Baseline ACS Presentation

Variable	Delipidated Group (n = 14)	Control Group (n = 12)	p Value
Total atheroma volume baseline, mm^3	229.3 ± 82.5	213.4 ± 104.0	0.594
Total atheroma volume follow-up, mm^3	217.1 ± 72.1	216.2 ± 102.8	0.629
Plaque burden baseline, %	45 ± 8	45 ± 8	0.899
Plaque burden follow-up, %	44 ± 9	45 ± 6	1.00
Mean max atheroma thickness baseline, mm	1.86 ± 0.34	1.7 ± 0.35	0.238
Mean max atheroma thickness follow-up, mm	1.78 ± 0.47	1.73 ± 0.36	0.859
Change in mean max atheroma thickness from baseline	−0.06 ± 0.17	0.01 ± 0.08	0.309
Atheroma volume follow-up, most diseased 10-mm subsegment	78.76 ± 22.05	80.49 ± 30.01	0.899
Change from baseline atheroma volume, most diseased 10-mm subsegment	−6.24 ± 17.94	−1.73 ± 11.21	0.494
Atheroma volume, least diseased 10-mm subsegment, baseline	50.1 ± 28.6	55.8 ± 22.3	0.494
Atheroma volume follow-up, least diseased 10-mm subsegment	49.02 ± 24.09	57.37 ± 24.12	0.325

ACS = acute coronary syndrome; IVUS = intravascular ultrasound.

only 5 weekly infusions of the preβ-like HDL apoA-I Milano/phospholipid complex suggests that acute intravenous HDL therapy may have the potential to reduce future clinical events in the ACS patient. The identification of the ABCA1 transporter as the major pathway for cholesterol efflux from cholesterol loaded cells, and the lipid poor preβ-HDL and not the αHDL as the ligand for the ABCA1 transporter, provided insight into the potential explanation for the marked effectiveness of the apoA-I Milano/phospholipid complex infusions in reducing coronary atherosclerosis.

The elucidation of the preβ-HDL/ABCA1 pathway for cholesterol efflux resulted in the quest for new approaches to obtain preβ-HDL for infusions in ACS patients. The development of the selective HDL delipidation of plasma with the conversion of the major plasma αHDL to preβ-like HDL provided a convenient source of preβ-like HDL (19). In the plasma delipidation process, cholesterol is selectively removed from αHDL particles with the formation of preβ-like HDL particles. The majority of circulating HDL is in the alpha form, with only about 5% occurring naturally in the preβ form. The overall selective HDL delipidation process involves removal of the plasma from the body, passing it through the LS PDS-2 delipidation device and then infusing the preβ-like HDL. In humans, 1 of the advantages is the autologous nature of the therapy, which potentially could be free of any toxicity to the patient. The selective HDL delipidation approach represented a novel concept since the process increased the plasma level of preβ-like HDL, the most effective form of HDL in cholesterol efflux without altering LDL or other lipoproteins.

Initial studies of the safety and efficacy of selective HDL delipidated plasma were performed in hyperlipidemic African Green monkeys. The selective delipidated HDL obtained from control monkey plasma was reinfused into 5 hyperlipidemic monkeys weekly for 12 weeks for a total of 123 infusions. Complete lipoprotein profile and clinical chemistry analyses were performed throughout the course of the infusions. The infusions were well tolerated, and laboratory analyses were not remarkable. Changes in aortic atherosclerosis associated with the selective HDL infusions were ascertained by IVUS quantitation of aortic lesions before and following the completion of the infusions in each animal (19). A 6.9% decrease ($p = 0.03$) in total atheroma volume was observed. Detailed HDL kinetic studies were also performed in the 5 monkeys to determine the metabolic fate of the infused lipid-poor preβ-HDL. Preβ-HDL had a plasma residence time of 8 ± 6 h and was converted entirely into large αHDL with a residence time of 13 to 14 h. These combined results indicated that the metabolic pathway of infused, selectively delipidated HDL was similar to the maturation of normal preβ-HDL to αHDL, and the infused preβ-HDL was associated with decreased atherosclerosis.

The current study presents the extension of this novel methodology of autologous selective delipidated HDL plasma infusions in ACS patients. This first-in-man trial demonstrates that treatment using the PDS-2 delipidation device with 7 weeks of delipidated HDL infusions is feasible and well tolerated by ACS subjects. Twenty-six of the 28 subjects (92.1%) completed all 10 clinic visits, demonstrating a high compliance rate. The plasma collection, delipidation, and reinfusion procedures demonstrated safety, ex-

Table 7 Intravascular Ultrasound Parameters

Variable	Delipidated Group (n = 14)	Control Group (n = 12)	p Value
Change in total atheroma volume, mm^3	−12.18 ± 36.75	2.80 ± 21.25	0.268
Change in plaque burden, %	−1.0 ± 4.0	0.0 ± 4.0	0.455
Change in most diseased 10-mm subsegment, atheroma volume, mm^3	−6.24 ± 17.94	−1.73 ± 11.21	0.594
Change in least diseased 10-mm subsegment, atheroma volume, mm^3	−1.10 ± 11.35	1.53 ± 11.70	0.417

Variables are mean ± SD.

Table 8 Comparison of the Changes in IVUS Parameters in Lipid Sciences Selective Delipidation Trial, ApoA-I Milano Trial, and REVERSAL Trial

Variable	Selective HDL Delipidation Trial (7 Weeks; n = 14)	ApoA-I Milano Trial* (5 Weeks; n = 36)	REVERSAL Trial† (18 Months; n = 253)
Change in total atheroma volume (mm^3)	−12.18 ± 36.75	−14.10 ± 39.50	−0.04 ± 31.80
Change in % atheroma-plaque burden	−1.0 ± 4.0	−1.1 ± 3.2	−0.6 ± 5.1
Change in 10-mm most diseased segment (mm^3)	−6.24 ± 17.94	−7.20 ± 12.60	−4.2 ± 12.8

Variables are mean ± SD. *Nissen et al. (18); †Nissen et al. (33).
HDL = high-density lipoprotein; IVUS = intravascular ultrasound.

cept for transient hypotension as the main adverse event during the plasma collection. This was most likely attributed to the plasmapheresis procedure and/or rate of plasma collection and was reversed following conservative fluid supplementation therapy in all patients. In addition, there were minimal differences seen in the blood chemistry (metabolic panel) and complete blood counts when assessing differences between pre-plasmapheresis and post-reinfusion per visit. These were considered minor changes, associated with no adverse events, and did not demonstrate any clinical sequelae across visits. Finally, although not significant, the exploratory IVUS analysis demonstrated a numeric reduction in atheroma volume in the delipidated group as compared with the placebo group.

It is of interest to compare the IVUS results in our selective delipidation trial utilizing the same IVUS methodology (when each patient serves as his or her own control, with paired comparisons performed between the baseline and end-of-study measures of atheroma burden) to the apoA-I Milano clinical trial. We found similar results in terms of the absolute atheroma volume reduction of -12.18 ± 36.75 mm^3 in our study compared with -14.10 ± 39.50 mm^3 reported in the apoA-I Milano infusion study (Table 8). It is also of interest to compare the results of the changes in atherosclerosis obtained with weekly infusions of preβ-HDL in apoA-I Milano and selective HDL delipidation trials with data obtained following the 18-month REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid Lowering) trial utilizing high-dose statin (80 mg atorvastatin) therapy (33) (Table 8). An almost 2-fold reduction in total atheroma volume was obtained with acute infusion therapy as compared with 18 months of statin therapy. These results, although not significant in our study in conjunction with the findings in the apoA-I Milano trial, add support to the concept that increasing preβ-HDL by acute HDL infusions may result in reduction in atheroma burden with stabilization of the plaque and decreased future clinical events in ACS patients.

This study has several limitations, particularly the small sample size, which limits interpretation of both the safety and the efficacy data. The majority of the patients were on statin therapy which could have also contributed to the efficacy of plaque regression. The study was not powered to detect any change in clinical events associated with the regression of atheroma volume due to the short interval of time of follow-up. Nevertheless, the delipidation process is repeatable and robust with a 28-fold increase in preβ-like HDL in the post-delipidated plasma when compared with the baseline pre-delipidated plasma. The IVUS findings can be a proof of concept only with regard to the consistent direction in reduction in the atheroma volume in this relatively short treatment interval, however, this extent of regression has never been described in patients treated with statins alone. These data on atherosclerosis, which are similar to the results reported in apoA-I Milano, are encouraging. Although this therapy is given only in an acute phase and for a short period of time, it is not clear whether acute regression of atherosclerotic burden will be associated with decreased clinical cardiovascular events. Additional clinical trials are required to determine whether acute HDL infusions will be associated with a decrease in clinical events.

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